PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY
(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference	**FOR FURTHER ACTION**
see form PCT/ISA/220	See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB2007/003486	12.09.2007	13.09.2006

International Patent Classification (IPC) or both national classification and IPC
INV. G01N33/557 G01N33/574

Applicant
ONCIMMUNE LTD

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☒ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:	Date of completion of this opinion	Authorized Officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	see form PCT/ISA/210	Lindberg, Pia Telephone No. +49 89 2399-7189

Form (PCT/ISA/237) (Cover Sheet) (April 2005)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/GB2007/003486

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed

   ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1 (b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43bis.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

      ☐ a sequence listing

      ☐ table(s) related to the sequence listing

   b. format of material:

      ☐ on paper

      ☐ in electronic form

   c. time of filing/furnishing:

      ☐ contained in the international application as filed.

      ☐ filed together with the international application in electronic form.

      ☐ furnished subsequently to this Authority for the purposes of search.

4. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

Box No. II Priority

1. ☒ The validity of the priority claim has not been considered because the International Searching Authority does not have in its possession a copy of the earlier application whose priority has been claimed or, where required, a translation of that earlier application. This opinion has nevertheless been established on the assumption that the relevant date (Rules 43*bis*.1 and 64.1) is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of

☐ the entire International application

☒ claims Nos. 1-36 (partially)

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international search *(specify)*:

☒ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. 1-36 (partially) are so unclear that no meaningful opinion could be formed *(specify)*:

see separate sheet

☒ the claims, or said claims Nos. 1-36 (partially) are so inadequately supported by the description that no meaningful opinion could be formed *(specify)*:

see separate sheet

☐ no International search report has been established for the whole application or for said claims Nos.

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:

- ☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
- ☐ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
- ☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rules 13*ter*.1(a) or (b).

☐ a meaningful opinion could not be formed without the tables related to the sequence listings; the applicant did not, within the prescribed time limit, furnish such tables in electronic form complying with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions, and such tables were not available to the International Searching Authority in a form and manner acceptable to it.

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in electronic form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See Supplemental Box for further details

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/GB2007/003486

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-36
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-36
Industrial applicability (IA)	Yes:	Claims	1-36
	No:	Claims	

2. Citations and explanations

see separate sheet

Box No. VI Certain documents cited

1. Certain published documents (Rules 43*bis*.1 and 70.10)

and / or

2. Non-written disclosures (Rules 43*bis*.1 and 70.9)

see form 210

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item I

Basis of the report

1.1 This opinion has been established on the basis of the international application in the language in which it was filed.

Re Item II

Priority

1.1 The validity of the priority document claimed has not been considered because the ISA does not have in its possession a copy of the translated priority document. This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1.1 Due to the non-compliance of the present application with respect to Articles 5 and 6 PCT, the search has been restricted to the basic principle of the application, namely to the method for the detection of a disease state or susceptibility of breast cancer by detecting autoantibodies against p53, c-myc, ECD6 or EDC6 3' fragment proteins by using the principle of an optimized immunoassay method based upon cross titration of an antigen and an antibody. See also Item V, 2.1-2.4

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:
D1: WO 99/58978 A (UNIV NOTTINGHAM [GB]; ROBERTSON JOHN FORSYTH

RUSSELL [GB]; GRAVES CATH) 18 November 1999 (1999-11-18)

D2: GB-A-2 395 270 (UNIV NOTTINGHAM [GB]) 19 May 2004 (2004-05-19)

D3: GREEN, J.A., ET AL: "Serum p53 Auto-antibodies: Incidence in Familial Breast Cancer" EUROPEAN JOURNAL OF CANCER, vol. 30A, no. 5, 1994, pages 580-584, XP002458469

D4: MICROBIX BIOSYSTEMS INC: "Antigen titration using the Microbix IgG ELISA"[Online] 2005, XP002458470 Retrieved from the Internet: URL:http://web.archive.org/web/20050526231 623/http://www.microbix.com/products/PDFs/ TB93-1AntigenTitrationusingtheMicrobixIgG+ ELISA.pdf>

D5: KUMAR, S., ET AL: "Standardisation and comparison of serial dilutions and single dilution enzyme linked immunosorbent assay (ELISA) using different antigenic preparations of the Babesia (Theileria) equi parasite" VETERINARY RESEARCH, vol. 34, no. 1, 2003, pages 71-83, XP002458471

D6: PARÉ J ET AL: "AN ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) FOR SEROLOGICAL DIAGNOSIS OF NEOSPORA SP. INFECTION IN CATTLE" JOURNAL OF VETERINARY DIAGNOSTIC INVESTIGATION, AAVLD, COLUMBIA, MO, US, vol. 7, 1995, pages 352-359, XP000944472 ISSN: 1040-6387

D7: GB-A-2 426 581 (UNIV NOTTINGHAM [GB]; ONC IMMUNE LTD [GB]) 29 November 2006 (2006-11-29)

D8: WO 2006/126008 A (ONC IMMUNE LTD [GB]; ROBERTSON JOHN FORSYTH RUSSELL [GB]; BARNES TONY) 30 November 2006 (2006-11-30)

D9: CHAPMAN, C., ET AL: "Autoantibodies in breast cancer: their use as an aid to early diagnosis" ANNALS OF ONCOLOGY, vol. 18, 7 March 2007 (2007-03-07), pages 868-873, XP002458472

D10: YANG, D-K., ET AL: "Development and evaluation of indirect ELISA for the detection of antibodies against Japanese encephalitis virus in swine" JOURNAL OF VETERINARY SCIENCE, vol. 7, no. 3, 30 September 2006 (2006-09-30), pages 271-275, XP002458473

1. Subject-matter of the application

1.1 The present application claims several methods of detecting an antibody in a test sample in a mammalian subject, wherein the antibody is either a biological marker of

a disease state or disease susceptibility or is directed to a foreign substance introduced in a mammalian subject, by preparing two or more different dilutions of the test sample and contacting the test sample dilutions with a plurality of different amounts of an antigen specific for the antibody. The specific binding between the antibody and antigen for each amount of antigen used is determined and a curve of the amount of the specific binding versus the amount of antigen for each test sample dilution is plotted. The presence of an antibody reactive with the antigen is indicated based upon the amount of specific binding or is indicated by a generally S shaped or sigmoid curve for at least two different dillutions of the test sample. Furthermore the applications claims the use of the methods in various applications relating to cancer and autoimmune diseases.

2. Clarity and Disclosure of the Invention

2.1 The present application is considered not to fulfil the requirements of Articles 5 and 6 PCT with respect to clarity and disclosure of the invention for the following reasons;

2.2 Independent claims 1, 5, 26 and 34 relate to methods of detecting a disease state or disease susceptibility in a mammalian subject by detecting an antibody in a test sample by preparing two or more different dillutions of the test sample and contacting the test sample dilutions with a plurality of different amounts of an antigen specific for the antibody. The amount of specific binding between the antibody and antigen is detected for each amount of antigen used, and a curve of the amount of specific binding versus the amount of antigen for each test sample dilution is plotted. The determination of the presence or absence of said disease or disease susceptibility is based upon the amount of specific binding or is indicated "by a generally S shaped or sigmoid curve".

The claims are not considered clear in the sense of Art. 6 PCT, as they fail to disclose at which amount of specific binding the presence or absence of the said disease state or disease susceptibility can be concluded or how the said "amount" is defined in terms of technical features.

2.3 Furthermore the claims are not considered to be supported within the whole scope as claimed, as the independent claims refer to the detection of any disease state or any

disease susceptibility, whereas the description of the application only discloses experimental data with primary breast cancer comprising the detection of antibodies against p53, ECD6 and ECD6 3' fragment proteins in primary breast cancer patients, wherein in all experiments the binding of the antigen (p53, c-myc, ECD6 or ECD6 3' fragment) with the antibody is measured using several dilutions of both the antigen and the antibody, Art. 6 PCT.

Moreover the application has not demonstrated that any of the tumor markers listed in claim 9 could be used in detecting a disease state or disease susceptibility (which amount of specific binding between antigen and antibody would reveal such conclusion?). Also the skilled person does not have any information about those specific markers, resulting to the presence of autoantibodies in a sample, that could be successfully used for the detection of any of the autoimmune diseases claimed in claim 23. Furthermore the skilled person does not have sufficient information about how to interpret the different curve forms resulting from plotting the "amount of specific binding" versus the amount of antigen for each test sample dilution, as the curve forms resulting from cancer and healthy control samples do not allow the assessment of a general pattern for the two sample groups, confer to figures 3A, B and 4 A, B. It seems impossible to distinguish disease state from healthy control based on the plotted curve form, Art. 5 PCT. Thus based on the disclosure of the application, it is not considered for the person skilled in the art to be possible to carry out the claimed invention within the whole scope of the application as claimed, Art. 5 and 6 PCT.

2.4 The search of the application was thus restricted accordingly to those parts of the applications that can be considered as supported in the sense of Art. 6 PCT, namely to a method for the detection of a disease state or susceptibility of breast cancer by detecting autoantibodies against p53, c-myc, ECD6 or EDC6 3' fragment proteins by using the principle of an optimized immunoassay method based upon cross titration of an antigen and an antibody.

3. Novelty and Inventive step

3.1 Claims 1-36 (partially) of the present application are considered novel in the sense of

Article 33(2) PCT, since the cited documents D1-D6 do not contain a method for detecting a disease state or susceptibility by detecting an antibody in a sample by determining the amount of specific binding between plurality of dilutions of an antibody (sample) with different amounts of an antigen specific for the antibody.

3.2 The present application is considered however not to fulfil the requirements of Art. 33(3) PCT with respect to inventive step for the following reasons;

The detection of a cancer disease state or disease susceptibility in a mammalian subject based on the detection of antibodies in a test sample is known from the prior art. For example **document D1** discloses a method for determining the immune response of a mammal to tumor antigens by detecting specific autoantibodies. The presence of complexes between tumor marker antigens and any autoantibodies to the antigens provide an indication of an immune response to a circulating tumor marker protein and the method can be used e.g. for the diagnosis of cancer, particularly for identifying new or recurrent cancer or for predicting outcome of treatments. The presence of such autoantibodies is detected by contacting a body fluid sample with a panel of two or more tumor marker antigens and determining the presence or absence of complexes between tumor marker antigens and autoantibodies, see D1 abstract and claims 1-72.

Equally **document D2** relates to a method of detecting tumor-associated autoantibodies by immunoassay. A sample is contacted with a tumor marker protein (MUC1, MUC16, c-myc, p53) and the presence of complexes formed between autoantibodies and the tumor marker antigen is detected. The method can be used for example for the detection or diagnosis of cancer, for monitoring the progress of cancer or other neoplastic disease in a patient or for identifying those subjects who are at increased risk of developing cancer, see D2 abstract and claims 1-38. **Document D3** discloses an ELISA assay for the detection of p53 autoantibodies in serum samples from breast cancer patients, see D3 page 581.

The subject-matter of the claimed method differs from the disclosure of any of D1-D3, considered as the closest prior art, in that the detection of complexes formed between antibodies and antigens is accomplished by measuring the complex formation using different sample (antibody) and antigen dilutions. This is to ensure that samples of

different kind of nature with respect to natural variations in antibody specificity/affinity and concentrations within individuals can be reliably measured. The objective technical problem may therefore been seen as the provision of a more specific and sensitive measuring method for samples of different characteristics to that presented in any of D1-D3.

3.3 The optimization of an assay method by testing multiple dilutions of the reagent components is well known from the prior art. For example **Document D4** discloses a laboratory protocol for an ELISA titration assay using human polyclonal antisera and anti-human IgG-Alkaline phosphatase conjugate for signal generation. D4 states that antigen titrations should be made to ensure optimal assay performance. The protocol discloses serial dilution of the antigen and an optimal dilution of the antiserum (antibody). The optimal dilution of the antisera is determined by performing dilutions of the sera against dilutions of the antigen. For interpretation of the results, the mean measured optical density values are plotted versus the antigen diluted. The optimal antigen dilution is then read from the graph, see D4 the whole document.

Furthermore **document D5** discloses standardisation and comparison of serial dilution and single dilution ELISA. Antibody titers of donkey sera were determined by serial dilution ELISA using three different antigens. The optimal dilution of the antigen and the conjugate was considered as the highest dilution of antigen/conjugate that gave a maximum contrast in optical density between known positive and negative serum dilutions. Moreover D5 states that different serum sample dilutions were required in order to obtain the best coefficient correlations when using different antigens, see D5 abstract, page 73, right-hand column, line 44- page 74, left-hand column, line 4 and page 76, right-hand column, second paragraph.

Document D6 refers to the optimization of an ELISA assay for the detection of antibodies to *Neospora* sp. in cattle. The optimal dilutions of the antigen, serum and conjugate were determined by titrations of the antigen (50-800ng/well), serum (1:50 to 1:800) and conjugate (1:5000 to 1:10000). As optimal dilutions for the assay were determined that combination of dilutions offering the best discrimination between positive and negative controls, see D6 page 353, right-hand column, line 1 - page 354, left-hand column, line 12.

3.4 As the optimization of ELISA assays with respect to used antigen and antibody concentrations (dilutions) is well known in the art, as illustrated by documents D4-D6, it is considered obvious for the person skilled in the art to optimize the method of any of D1-D3 for better sensitivity by applying such principle of preparing several antigen and antibody dilutions for optimal measurement conditions.

The subject-matter of the application referring to claims 1-36 (partially) is thus not considered inventive over the disclosure of any of D1-D3 combined with any of D4-D6 in the sense of Art. 33(3) PCT.

4. Industrial applicability

4.1 Claims 1-36 (partially) of the present application are considered industrially applicable and thus meet the criteria of Article 33(4) PCT.

Re Item VI
Certain documents cited

D7: GB-A-2 426 581 (UNIV NOTTINGHAM [GB]; ONC IMMUNE LTD [GB]) 29 November 2006 (2006-11-29)

D8: WO 2006/126008 A (ONC IMMUNE LTD [GB]; ROBERTSON JOHN FORSYTH RUSSELL [GB]; BARNES TONY) 30 November 2006 (2006-11-30)

D9: CHAPMAN, C., ET AL: "Autoantibodies in breast cancer: their use as an aid to early diagnosis" ANNALS OF ONCOLOGY, vol. 18, 7 March 2007 (2007-03-07), pages 868-873, XP002458472

D10: YANG, D-K., ET AL: "Development and evaluation of indirect ELISA for the detection of antibodies against Japanese encephalitis virus in swine" JOURNAL OF VETERINARY SCIENCE, vol. 7, no. 3, 30 September 2006 (2006-09-30), pages 271-275, XP002458473

1.1 The above cited documents (marked as D7-D10 under Item V) may become relevant

and fall within the scope of the application in case the priority of the application turns out not to be valid. Documents D7-D8 disclose methods for detecting disease state or disease susceptibility by detecting an antibody in a test sample wherein the test sample is contacted with a plurality of different amounts of antigen specific for the antibody. Document D9 refers to the detection of autoantibodies in breast cancer and D10 to an optimized ELISA method for the detection of antibodies in serum samples.

Re Item VIII

Certain observations on the international application

1.1 The present application is considered not to comply with the requirements of Articles 5 and 6 PCT, see Item V, 2.1-2.4.

Possible steps after receipt of the international search report (ISR) and written opinion of the International Searching Authority (WO-ISA)

General information	For all international applications filed on or after 01/01/2004 the competent ISA will establish an ISR. It is accompanied by the WO-ISA. Unlike the former written opinion of the IPEA (Rule 66.2 PCT), the WO-ISA is not meant to be responded to, but to be taken into consideration for further procedural steps. This document explains about the possibilities.
Amending claims under Art. 19 PCT	Within 2 months after the date of mailing of the ISR and the WO-ISA the applicant may file amended claims under Art. 19 PCT directly with the International Bureau of WIPO. The PCT reform of 2004 did not change this procedure. For further information please see Rule 46 PCT as well as form PCT/ISA/220 and the corresponding Notes to form PCT/ISA/220.
Filing a demand for international preliminary examination	In principle, the WO-ISA will be considered as the written opinion of the IPEA. This should, in many cases, make it unnecessary to file a demand for international preliminary examination. If the applicant nevertheless wishes to file a demand this must be done before expiry of 3 months after the date of mailing of the ISR/WO-ISA or 22 months after priority date, whichever expires later (Rule 54bis PCT). Amendments under Art. 34 PCT can be filed with the IPEA as before, normally at the same time as filing the demand (Rule 66.1 (b) PCT). If a demand for international preliminary examination is filed and no comments/amendments have been received the WO-ISA will be transformed by the IPEA into an IPRP (International Preliminary Report on Patentability) which would merely reflect the content of the WO-ISA. The demand can still be withdrawn (Art. 37 PCT).
Filing informal comments	After receipt of the ISR/WO-ISA the applicant may file informal comments on the WO-ISA directly with the International Bureau of WIPO. These will be communicated to the designated Offices together with the IPRP (International Preliminary Report on Patentability) at 30 months from the priority date. Please also refer to the next box.
End of the international phase	At the end of the international phase the International Bureau of WIPO will transform the WO-ISA or, if a demand was filed, the written opinion of the IPEA into the IPRP, which will then be transmitted together with possible informal comments to the designated Offices. The IPRP replaces the former IPER (international preliminary examination report).
Relevant PCT Rules and more information	Rule 43 PCT, Rule 43bis PCT, Rule 44 PCT, Rule 44bis PCT, PCT Newsletter 12/2003, OJ 11/2003, OJ 12/2003

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